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Appendix A

Patent and Trademark

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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53 (b)(2).

Docket Number	BT-4522	Type a plus sign (+) inside this box ->	+

INVENTOR(s)/APPLICANT(s)

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See attached sheet for additional inventors			

TITLE OF THE INVENTION (280 characters max)

THERMAL CYCLER FOR PCR

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ENCLOSED APPLICATION PARTS *(check all that apply)*

X	Specification	*Number of Pages*	17	☐	Small Entity Statement
X	Drawing(s)	*Number of Sheets*	12	☐	Other (specify)

METHOD OF PAYMENT *(check one)*

☐	A check or money order is enclosed to cover the Provisional filing fees	PROVISIONAL FILING FEE AMOUNT ($)	150
+	The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number: 16-1335		

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: ____________

Respectfully submitted,

SIGNATURE David Aker   Date 5/8/97

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Provisional
In re application of: John G. Atwood et al.
Serial No: 0 / Docket #BT-4522 Group No.:
Filed: Concurrently herewith Examiner:
For: THERMAL CYCLER FOR PCR

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THERMAL CYCLER FOR PCR

FIELD OF THE INVENTION

This invention pertains to the field of computer controlled instruments for performing the Polymerase Chain Reaction (PCR). More particularly, the invention pertains to automated instruments that perform the reaction simultaneously on many samples and produce very precise results by using thermal cycling.

BACKGROUND OF THE INVENTION

The background of the invention is substantially as stated in U.S. Patent No. 5,475,610 which is herein incorporated by reference.

SUMMARY OF THE INVENTION

According to the invention, there is provided a sample block of novel design with low thermal mass for rapid temperature excursions. The sample block is manufactured from silver for uniform overall heat distribution and has a bottom plate for uniform lateral heat distribution. In addition, to further offset heat losses and resulting temperature gradients from the center to the edges, a center pin is used as a conducting path to the heat sink.

The invention also provides a novel method and apparatus for achieving rapid heating and cooling using thermoelectric devices. These devices are precisely matched to each other. They are constructed using die cut alumina on both sides to minimize thermal expansion and contraction. The alumina facing the sample block is thinner to minimize the heat load. The devices are constructed of Bismuth Telluride (BT) using specific dimensions to achieve matched heating and cooling rates. They are designed using minimal copper thicknesses internally to further reduce their heat load characteristics and are assembled using a specific high temperature solder in specified quantities.

The invention is also directed to a novel heatsink design having a specific fin arrangement. The heatsink is constructed with a perimeter trench to limit heat conduction and losses from its

edges. Furthermore, the heatsink has an attached variable speed fan to assist in maintaining a constant temperature or in cooling.

The invention is also directed to a clamping mechanism to hold the sample block to the heat sink with the thermoelectric devices positioned in between. The mechanism is designed to provide evenly distributed pressure with a minimal heat load. The design allows the use of thermal grease as an interface among the sample block, the thermoelectric devices and the heatsink.

There is also provided a perimeter heater to minimize the thermal non-uniformity across the sample block. The perimeter heater is positioned around the sample block to counter the heat loss from the edges. Power is applied to heater in proportion to the sample block temperature with more power applied when the sample block is at higher temperatures and less power applied when the sample block is at lower temperatures.

There is also provided a heated cover, designed to keep the sample tubes closed during cycling and to heat the upper portion of the tubes to prevent condensation. The heated cover applies pressure on the sample tube cap perimeter to avoid distorting the cap's optical qualities. The cover is self-aligning, using a skirt which mates with the sample tube tray.

The invention is also directed to a method and apparatus for determining an ideal temperature ramp rate which is determined to take advantage of sample block temperature overshoots and undershoots in order to minimize cycle time.

The invention also include a method and apparatus for linearizing the thermal power output from the thermal electric cooling devices for both linear and non linear ramp rates and to achieve linear temperature control.

The invention may be used with calibration diagnostics which compensate for variations in the performance of thermoelectric devices such that all instruments perform identically. The thermal characteristics and performance of the assembly comprised of the sample block, thermal electric devices and heatsink, is stored in an on-board memory device, allowing the assembly to be moved to another instrument and behave the same way.

The invention further includes a method and apparatus for measuring the AC resistance of the thermoelectric devices to provide early indications of device failures.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a cross sectional view of a portion of the sample block according to the invention.

Figure 2 is an enlarged, isometric view of a thermal electric device constructed according to the invention.

Figure 3 is a cut-away, isometric view of the heatsink according to the invention.

Figure 4 is an exploded view of the assembly consisting of a sample block, thermoelectric devices and heatsink.

Figure 5 is an isometric view of the novel heated cover in accordance with the invention .

Figure 6 is a chart depicting the Up Ramp (heating rate) vs. Power.

Figure 7 is a chart depicting the Down Ramp (cooling rate) vs. Power.

Figure 8 is a chart for predicting and compensating for temperature overshoots and undershoots in accordance with the invention.

Figure 9 is a block diagram of the AC resistance measurement means of the invention.

Figure 10 shows a perimeter heater and its approximate location surrounding the sample block.

Figure 11 shows a detailed view of the perimeter heater.

Figure 12 shows the power applied to the perimeter heater as a function of the temperature of the sample block

DETAILED DESCRIPTION OF THE INVENTION

Generally, in the case of PCR, it is desirable to change the sample temperature between the required temperatures in the cycle as quickly as possible for several reasons. First the chemical reaction has an optimum temperature for each of it's stages and as such less time spent at non-optimum temperatures means a better chemical result is achieved. Secondly a minimum time is usually required at any given setpoint which sets a minimum cycle time for each protocol and any time spent in transition between setpoints adds to this minimum time. Since the number of cycles is usually quite large, this transition time can significantly add to the total time needed to complete the amplification.

The absolute temperature that each reaction tube attains during each step of the protocol is critical to the yield of product. As the products are frequently subjected to quantitation, the

product yield in each tube must be as uniform as possible and therefore both the steady-state and dynamic thermal uniformity must be uniform across the block.

Heat-pumping into and out of the samples is accomplished by using thermoelectric devices. These are constructed of pellets of n-type and p-type bismuth telluride connected alternately in series. The interconnections between the pellets is made with copper which is bonded to a substrate, usually a ceramic (typically alumina).

The amount of heat-pumping required is dependent on the thermal load and the ramp rate, that is, the rate at which the temperature is required to change. The sample tube geometry and sample volumes are not variables as the sample tubes are established as an industry standard, fitting into many other types of instruments such as centrifuges, and the sample volume is defined by user need. Therefore the design variables primarily affect the sample block, thermoelectric devices, heatsink, fan and the thermal interface media between the thermoelectric devices and both the heatsink and the sample block.

The block geometry must also meet the necessary thermal uniformity requirements because it is the primary contributor to lateral conduction and therefore evens out any variation in thermal uniformity of the thermoelectric coolers themselves. The conflicting requirements of rapid ramp rates (indicating low thermal mass) and high lateral conduction (indicating a large material mass) are met by concentrating the bulk of the block structure in a base plate, and minimizing the thermal mass of the upper portion of the block which holds the sample tubes. The optimal material for block fabrication is pure silver which has relatively low thermal mass and very good thermal conduction. Silver also lends itself well to electroforming. In practice the optimal block geometry has a light electroformed upper portion to hold the sample tubes fixed to a relatively thick base plate which provides lateral conduction. The thermal mass of the block is concentrated in the base plate where the material contributes the most to thermal uniformity. The electroformed portion of the block has a minimum thickness which is defined by two parameters. First the material cannot be so thin as to make it too delicate for normal handling. Second the wall thickness is required to conduct heat out of the upper regions of the sample tube. The sample itself is circulated by convection inside the tube and it's temperature is relatively uniform vertically, but good thermal conductivity between the tube walls and the base plate increases the effective surface area available for conduction of heat between the sample and the base plate. The

base plate thickness has a minimum value defined by lateral conduction requirements which is a function of the thermal uniformity of the thermoelectric coolers and structural rigidity.

Another contributor to the thermal mass is the alumina ceramic layers which form part of the structure of the thermoelectric cooler itself. There are two alumina layers in the construction of the thermoelectric cooler, one on the sample block side and another on the heatsink side. The layer on the side of the sample block forms a significant part of the thermal load. The thickness of the layer should be minimized as much as possible, in this case the practical limit of thinness for the alumina thickness is defined by the manufacturing requirements of thermoelectric cooler fabrication. This particular layer of ceramic could in principal be replaced by a different layer altogether such a thin sheet of Kapton which would reduce the thermal mass even more, but at the present time although coolers are available with this structure, reliability is unproven. It is anticipated that once the technology has been developed further, then a cooler of such a design may be preferred. However, the thin alumina layer also contributes to system reliability.

The copper conductors within the cooler are a significant thermal load and are not overlooked in the design of the system. The thickness of the copper traces is defined by the requirement to carry current through the device, and once the current is known the copper thickness can be calculated.

Sample Block

Figure 1 shows a cross sectional view of a portion of the sample block which typically has 96 wells, each for receiving a sample vial. The sample block is constructed of silver and comprises an electroformed sample well portion **20** and a base plate **22** portion. The base plate **22** provides lateral conduction to compensate for any difference in the thermal power output across the surface of each individual TED and for differences from one TED to another.

There are always boundary losses in any thermal system. In a rectangular configuration there is more heat loss in the corners One solution is to use a round sample block, but the microtiter tray format that is in common usage is rectangular and this must be used to retain compatibility with other existing equipment. Once the edge effects have been eliminated using all standard means, such as insulation etc., there remains a tendency for the center of the sample block to be warmer than the corners. Typically it is this temperature difference that defines the

thermal uniformity of the sample block. In this system the center temperature has been reduced by providing a small thermal connection from the center of the sample block to the heat sink. By using a pin **24** which acts as a "heat leak" in the center of the sample block, the temperature gradient across the sample block can be reduced to an acceptable level. The amount of conduction required is quite small and a 1.5mm diameter stainless steel pin has been found to be sufficient.

Thermal Electric Devices (TED's)

Thermal uniformity of the sample block is critical to PCR performance. One of the most significant factors affecting the uniformity is variations in the TED performance between devices. The most difficult point at which to achieve good uniformity is during a hold cycle far from ambient. In practice this is in a steady state hold at approximately 95°C. The TED devices are matched under these conditions to make a set of devices for each heatsink assembly which individually produce the same temperature for a given input current. The TEDs are matched to within 0.2°C in any given set, this value being derived from the maximum discrepancy that can be rectified by the lateral conduction of the sample block baseplate.

Figure 2 shows a typical thermal electric device. The alumina layer **26** that forms the outer wall of the TED, expands and contracts during temperature cycling at a different rate than the sample block. The motion of the alumina is transmitted directly to the solder **28** connecting the internal bismuth telluride pellets **30**. This motion can be reduced dramatically by cutting the alumina into small pieces **32** called die so that the field of expansion is small. The minimum size of the die is defined by the copper traces required to carry current through the TED and factored to retain some strength in the device for handling purposes.

Using a thin alumina layer in the TED (of the order of 0.058 cm) not only reduces the thermal load but also means that for a given required heat pumping rate the temperature that the ends of the pellet reaches is reduced due to the increase in k. This enhances reliability by reducing the thermal stress on the solder joint.

Generally in PCR the reaction temperatures are in the range 35 to 96°C i.e. cycling occurs above ambient. In the most important case the block is being heated or cooled between two above ambient temperatures where the flow of heat due to conduction usually occurs from the

block to the heat sink. The key to optimizing the system cycle time, given an optimized block configuration, is to balance the boost to the ramp rate when cooling provided by the conduction against the boost provided to the heating ramp rate by the joule effect.

If the cross-section of the Bismuth Telluride pellets in a given TED were considered constant, the heating ramp rate would be increased by increasing the length of the pellet. This is because the conduction path through the TED would be made longer thereby decreasing **k**. This also has the effect of reducing the current required to hold at a given block temperature in the steady state. During the down ramp, i.e. cooling the block, the decreased **k** means that the conduction contribution will be reduced and so the down ramp rate will be reduced.

Conversely, if the length of the Bismuth Telluride pellet were to be decreased for a given cross-section, then **k** would be increased. This would increase the current required to hold at an elevated temperature in the steady state and would increase the cooling ramp rate. Heating ramp rates would be reduced as a larger portion of the heat in the block would be conducted directly to the heat sink. Decreasing the Bismuth Telluride pellet length also increases the holding power required for a given temperature due to the losses through the TEDs and reduces the thermal load increasing the maximum possible ramp rate for given power. Therefore the optimized TED can be derived by adjusting the length of the Bismuth Telluride pellets until the heating rate matches the cooling rate.

The ratio l:A for the pellets also defines the resistance of the device i.e.

$$R=n.r.(l/A)$$

where n is the number of pellets, r is the resistivity of the Bismuth Telluride being used, l is the length of the pellet and A is the cross-sectional area.

The resistance must be measured as an AC resistance because of the Seebeck effect. Because the geometry defines the resistance of the device another design boundary is encountered in that the device must use a cost effective current to voltage ratio because too high a current requirement pushes up the cost of the amplifier. The balanced solution for the silver electroformed block described above is :

Pellet length = 1.27mm

Pellet cross-sectional area = 5.95mm^2

If the thermal cycler was to be used as part of another instrument, e.g. integrated with detection technology, then it may be more convenient to use a different current source which would lead to a modified TED geometry.

The TEDs are soldered together. Excess solder can wick up the side of the Bismuth Telluride pellets and where this occurs k is increased which results in a local cold spot [also called mild spot]. These are reduced in number and severity by application of the minimum amount of solder during the assembly process of the TED. It is also necessary to ensure that the solder used to attach the connecting wires to the TED does not contact the pellet for the same reason.

High temperature solder has been shown to not only have improved high temperature performance but it is also generally more resistant to failure by stress reversals and hence is most appropriate in this application. The solder used in this invention may be of the type as described in U.S. Patent 5,441,576.

Heatsink

Figure 3 shows the heatsink **34** with the sample block **36** mounted on top. The thermal mass of the heat sink is considerably larger than the thermal mass of the sample block and samples combined. The sample block plus samples together have a thermal mass of approximately 100 joules /K and that of the heat sink is approximately 900 Joules/K. This means that the sample block clearly changes temperature much faster than the heat sink for a given amount of heat pumped. In addition the heat sink temperature is controlled with a variable speed fan. The temperature of the heat sink is measured by a thermistor **38** placed in a recess **40** within the heatsink and the fan speed is varied such as to hold the heat sink at approximately 45°C in the normal PCR cycling temperature range, where maintaining a stable heat sink temperature improves the repeatability of system performance. However, the heat sink temperature is changed under some other conditions. When the block temperature is set to a value below ambient then the heat sink is best set to the coolest achievable temperature to reduce system power consumption

and optimize block thermal uniformity. This is accomplished simply by turning the fan up to full speed.

The heat sink temperature measurement is also used by the TED control algorithm described below in linearizing the thermal output power from the TEDs.

The heatsink temperature uniformity is reflected in the uniformity of the block temperature. Typically the heatsink is warmer in the middle than it is at the edges and this adds to other effects that lead to the corners of the block being the coldest. This is controlled by removing fins from the corners of the heatsink in those regions **42**.

A trench **44** is cut into the heat sink outside the perimeter of the TED area which limits the conduction of heat and decreases edge losses from the area.

Clamping Mechanism

TED manufacturers recommend that the TEDs be held under pressure as this has been shown to improve life-expectancy. The pressure that is recommended varies from manufacturer to manufacturer but is in the range of 30 to 100 psi. Higher pressures are acceptable, up to 300 psi for non-cycling applications. The pressure recommended is often defined by the thermal interface media selected.

There are many thermal interface media available in sheet form which can be used to act as a compliant layer on each side of the TEDs, but it has been demonstrated that thermal grease gives far superior thermal performance for this application. Thermal grease does not require high pressure to ensure good thermal contact has been made, unlike other compliant sheets which have been shown to require 30 psi or more even in optimal conditions. Also thermal grease does act as effective lubricant between the expanding and contracting silver block and the TED surface, thus enhancing life-expectancy. Thermalcote II thermal grease manufactured by Thermalloy, Inc. may be used.

Because the silver block is relatively flexible and soft it cannot transmit clamping pressure laterally very effectively, but the low pressure required by the grease reduces the requirement to transmit this force. Figure 4 shows an exploded view of the assembly with the best embodiment of the clamping mechanism **46**. Because of the open honeycomb structure of the silver electroform it is possible to apply clamping pressure in the body of the block by inserting clamp

"fingers" **48** into the block structure thereby applying the pressure more evenly that an edge clamping scheme would. These fingers apply pressure at a local point to minimize the contact area between the mass of the clamp and the sample block so that the clamp does not add significantly to the thermal load. The clamps are molded from a glass filled plastic which has the necessary rigidity for this application. The pressure is applied by deforming the fingers with respect to mounting posts **50** which are held flush to the surface of the heat sink with screws **52**. This scheme eliminates the necessity to set the pressure with adjustment screws as the clamps can simply be tightened down.

Pressure distribution should be as even as possible to ensure that the full area of the TEDs are in good thermal contact with the block and, most importantly, the heatsink. Good thermal contact reduces local thermal stresses on the TEDs. Even pressure is achieved as described above.

Perimeter Heater

In order to bring the temperature uniformity across the sample block to approximately ±0.2°C, a perimeter heater is positioned around the sample block to eliminate heat losses from its edges. Preferably, the heater is a film type, having low mass with inside dimensions slightly larger than the sample block. Figure 10 shows the perimeter heater **74** and its approximate location surrounding the sample block **36**. The heater is not fastened in place, it is simply positioned in the air around the perimeter of the sample block in order to warm the air in the immediate vicinity.

Figure 11 shows a detailed view of the perimeter heater **74**. The heater is rectangular as determined by the dimensions of the sample block and is manufactured so that it has separate power densities in specific areas to reflect the varying amounts of heat loss around the perimeter of the block. Matching lower power density areas **76** (0.73 W/in^2) are located in the center portions of the short sides of the rectangle and matching higher power density areas **78** (1.3 W/in^2) are located in the longer sides, extending into the shorter sides.

As shown in Figure 12, the power applied to the perimeter heater is regulated to correspond to the temperature of the sample block, with more power applied to the heater at higher block temperatures and less applied at lower block temperatures.

Heated Cover:

Figure 5 shows the heated cover. The heated cover applies pressure to the sample vial caps to ensure that they remain tightly closed when the sample is heated. The cover is heated to a temperature above that of the sample to ensure that the liquid does not condense onto the tube cap and instead remains in the bottom of the tube where thermal cycling occurs. This is described in United States Patent No. 5,475,610, mentioned above. The heated platen **54** in the present invention does not press on the dome of the cap but instead presses on the cap perimeter. The platen has a surface shaped in this manner so that optical caps are not modified by the application of pressure. Thus, tubes that have been cycled can be directly transferred to an optical reader without the need to change the cap.

Because the heated platen has recesses **56** in it to clear the cap domes, there is a need to align the plate to the tube positions before applying pressure to avoid damage to the tubes. This is accomplished by use of a "skirt" **58** around the perimeter of the platen which aligns to the microtiter tray before the plate touches the tube caps.

Determining the Ideal Ramp Rate:

The optimized ramp rate has been empirically determined to be 4°C/sec. Any system which has a higher block ramp rate than this cannot fully utilize the benefits of overshoots and consequently achieves an insignificant reduction in cycle time.

Figure 6 is a chart depicting the Up Ramp (heating rate) vs. Power and Figure 7 is a chart depicting the Down Ramp (cooling rate) vs. Power.

When heating the block to a temperature above ambient the Joule heating and the Seebeck heat pumping both act to heat the block against conduction. When cooling the block between two temperatures above ambient, the Seebeck heat pumping and conduction act against the Joule heating. During cooling, significant power is required to hold the block temperature steady against the flow of heat out of the block by conduction. Therefore even with zero power applied, the block will cool at a significant rate. As the current is increased, the Seebeck effect increases the cooling obtained. However as the current is increased further the joule effect, which is proportional to the square of the current, quickly starts to take over acting against the Seebeck cooling. Therefore a point is reached where applying additional power acts against the required

effect of cooling. In the heating mode these two effects act together against conduction and no ceiling is reached. In practice the heating power vs. input current is approximately linear. This is why the design centers around meeting the cooling rate requirements; the heating rate can always be achieved by the application of more power.

Linearizing the Output of the TED's

Equation 1 describes the total heat flow from the cold side of a thermal electric cooler.

$$0 = 1/2 * R\left(t_{avg}\right) * I^2 + t_C * S(t_{avg}) * I - \left(k\left(t_{avg}\right) * \left(t_c - t_h\right) + Q_c\right) \qquad \textbf{Equation 1}$$

where

t_c	= cold side temperature of cooler
t_h	= hot side temperature of cooler
t_{avg}	= average of t_c and t_h
R(t)	= electrical resistance of cooler as a function of temperature
S(t)	= Seebeck coefficient of the cooler as a function of temperature
K(t)	= Conductance of cooler as function of temperature
I	= electrical current applied to cooler
Q_c	= total heat flow from the cold side of the cooler

Given a desired heat flow, Q_c, and the hot and cold side temperatures, t_c and t_h, equation 1 is solved for I, the current required to produce Q_c. The solution of this equation is used for three purposes:

1) To achieve linear temperature transitions or ramps.

For linear temperature transitions, constant thermal power is required. To maintain constant thermal power when temperatures t_c and t_h are changing, I in equation 1 is solved for periodically and the result is applied to the coolers. To compensate for errors a proportional integral derivative (PID) control loop may be applied where:

Error input to PID = Setpoint Rate - Actual Rate

and Output from the PID is interpreted as percent Q

2) To achieve a linear PID temperature setpoint control algorithm over the desired temperature range.

Input to the PID control is the error signal t_c - SetPoint.

Output from the PID control is interpreted as a % of Q_{max}.

Equation 1 is used to determine the current value, **I**, which will result in the % of Q_{max} output by the PID control, under the current temperature conditions.

3) To achieve non-linear temperature transitions or ramps where temperature transitions are defined by the derivative of temperature with respect to time, **dT/dt**, as a function of block temperature.

This function is approximated by a table containing Block T, dT/dt data points in 5 C increments for cooling and by a linear equation for heating. The small effect of sample mass on dT/dt profiles, although measurable, is ignored. Knowing the total thermal mass, **MCP (Joules/K)**, involved during temperature transitions, the amount of thermal power, **Q (Joules/sec)**, required to achieve the desired rate profile, **dT/dt (K/sec)**, is given at any temperature by the following equation:

$$Q = MCP * dT/dt$$

The solution to equation 1 is used to determine the current value, **I**, which will result in the desired **Q** under the current temperature conditions. This process is repeated periodically during temperature transitions.

Controlling overshoot and undershoot

Figure 8 is a chart for predicting and compensating for temperature overshoots and undershoots. An optimized system uses a symmetrical bi-polar power amplifier to drive the TEDs and therefore there is advantage in balancing the TED geometry such that for a given maximum current in either polarity, the heating rate matches the cooling rate. This is because there is a practical limit to the ramp rates and hence cycle times that can be achieved. The sample has a time constant with respect to the block temperature that is a function of the sample tube and

tube geometry which, being an industry standard, cannot be reduced. This means that even if the sample tube wall temperature is changed as a step function e.g. by immersion in a water bath, the sample will have a finite ramp time as the sample temperature exponentially approaches the set point. This can be avoided in a system where the block is controlled dynamically by the use of programmed overshoots. This means that the block temperature is driven beyond the setpoint and back again as a means of minimizing the time taken for the sample to reach setpoint. As the possible ramp-rate increase the over shoot required to optimize the time taken for the sample to reach setpoint gets larger and a practical limit is soon reached. This occurs because although the average sample temperature does not overshoot the setpoint, the boundary liquid layer in the tube does overshoot to some extent. When cooling to the priming temperature, too great an over shoot can result in non-specific priming. Therefore the best advantage is to be gained in a system which utilizes this maximum ramp rate combined with optimized overshoots that are symmetrical on both up and down ramps.

Calibration Diagnostics:

The control software includes calibration diagnostics which permit variation in the performance of thermoelectric coolers from instrument to instrument to be compensated for so that all instruments perform identically. The sample block, TEDs and heatsink are assembled together and clamped using the clamping mechanism described above. The assembly is then ramped through a series of known temperature profiles during which its actual performance is compared to the specified performance. Adjustments are made to the power supplied to the TEDs and the process is repeated until actual performance matches the specification. The thermal characteristics obtained during this characterization process are then stored in a memory device residing on the assembly. This allows the block assembly to be moved from instrument to instrument and still perform within specifications.

AC Resistance Measurement:

The typical failure mode for the TEDs is an increase in resistance causes by a fatigue failure in a solder joint. This results in an increase in the temperature of that node which stresses the node further and rapidly pushes that node to catastrophic failure. It has been determined

empirically that devices that exhibit an increase in AC resistance of around 5% after about 20,000 to 50,000 temperature cycles will shortly fail. If the AC resistance of the TEDs can be monitored by the instrument, imminent failures can be detected and corrected before the device in question causes a thermal uniformity problem.

This embodiment automates the actual measurement using a feedback control system and eliminates the need to remove the TED device from the unit. The control system compensates for the temperature difference between the two surfaces of the TED device caused by the heat sink attached to one side and the sample block attached to the other. The control system causes the TED device to equalize its two surface temperatures and then the AC resistance measurement is made. The micro-controller performs a polynomial calculation at the referenced time of the AC measurement to compensate for ambient temperature error.

Figure 9 shows the sample block **36**, TED **60** and heatsink **34** interfaced with the system microcontroller **62** and bipolar power amplifier **64**. The temperature sensor already present in the heatsink **38** and an additional temperature sensor attached to the sample block **66** are utilized to determine the temperature differential of the surfaces of the TED.

The bipolar power amplifier supplies current in two directions to the device. Current in one direction heats the sample block and current in the other direction cools the sample block. The bipolar power amplifier also has signal conditioning capability to measure the AC voltage and AC current supplied to the TED. A band pass filter **68** is incorporated into the signal conditioning to separate an AC measurement signal from the steady state signal that produces a null condition for the temperature difference across the TED device.

The micro-controller incorporates the necessary capability to process the measurement information and perform the feedback in real time. It also stores the time history of the AC resistance and the number of temperature cycles of the TED and displays the information to the operator on the display **70**. The AC measurement is normally done during initial turn on. However, it can be activated when self diagnostics are invoked by the operator using the keypad **72**. An analog to digital and digital to analog converter along with signal conditioning for the temperature sensors and AC resistance measurement is also integrated into the micro-controller in order for it to perform its digital signal processing.

Sealing the TED Area from the Environment.

The TEDs are protected from moisture in the environment by seals and the chamber is kept dry with the use of a drying agent such as silica gel. The seal connects from the silver electroform to the surrounding support and as such adds to the edge losses from the block. These losses are minimized by the use of a membrane seal that is bonded in place rather than a compression seal. Bonding the seal in place allows a thin seal to be used which minimizes the cross-sectional area that is available for heat transfer out of the block.

ABSTRACT

An instrument for performing highly accurate PCR employing a sample block in microtiter tray format. The sample block temperature is changed exclusively by thermoelectric coolers controlled by a computer. The sample block is of low thermal mass and this sits directly on the thermoelectric coolers and the coolers sit on-top of a swaged fin heat sink. The heat sink temperature is controlled by a continuously variable fan positioned beneath the heat sink on the opposite side to the thermoelectric coolers. The sample temperature is calculated instead of measured as in U.S. Patent No. 5,475,610. A perimeter heater is used to improve the thermal uniformity across the sample block to approximately ±0.2°C. A heated platen pushes down onto the tube caps to apply a minimum acceptable force for seating the tubes into the block, ensuring good thermal contact with the block. The force is applied about the periphery of the tube caps to prevent distortion of the caps during thermal cycling. The platen is heated to provided thermal isolation from ambient conditions and to prevent evaporation from the surface of the sample into the upper portion of the sample tube. A control algorithm manipulates the current supplied to the thermoelectric coolers such that the dynamic thermal performance of the block can be controlled so that pre-defined thermal profiles for the sample temperature can be executed. The control software includes calibration diagnostics which permit variation in the performance of thermoelectric coolers from instrument to instrument to be compensated for such that all instruments perform identically. The block/heat sink assembly can be changed to another of the same or different design. The block assembly carries the necessary information required to characterize it's own performance in an on-board memory device and this allows the block assembly to be moved to another instrument and behave the same way. The instrument has a graphical user interface. The instrument design maximizes the life-expectancy of the thermoelectric coolers.

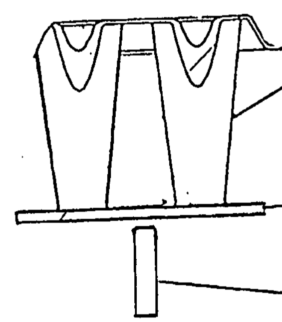


FIGURE 1

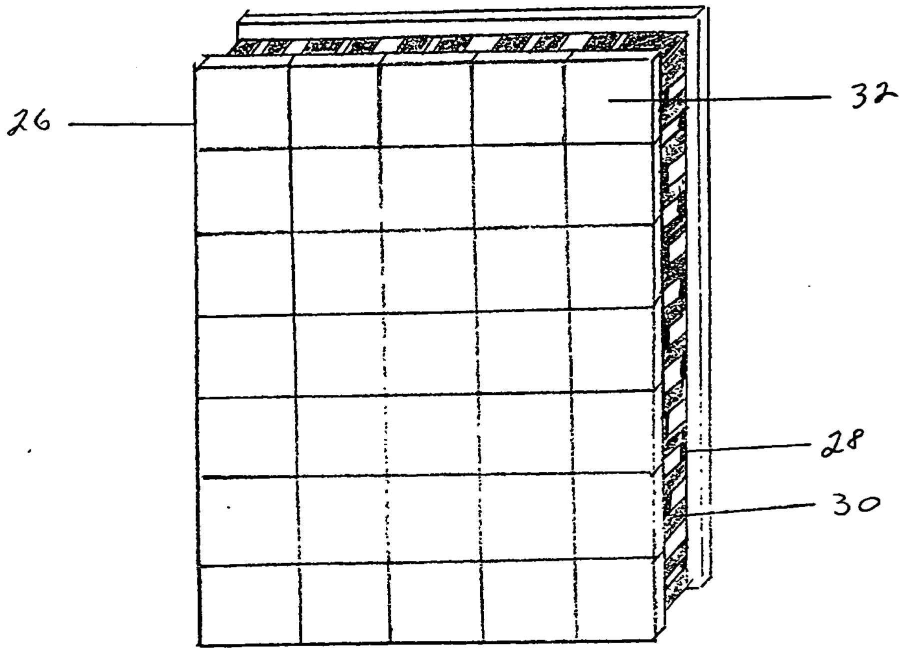


FIGURE 2

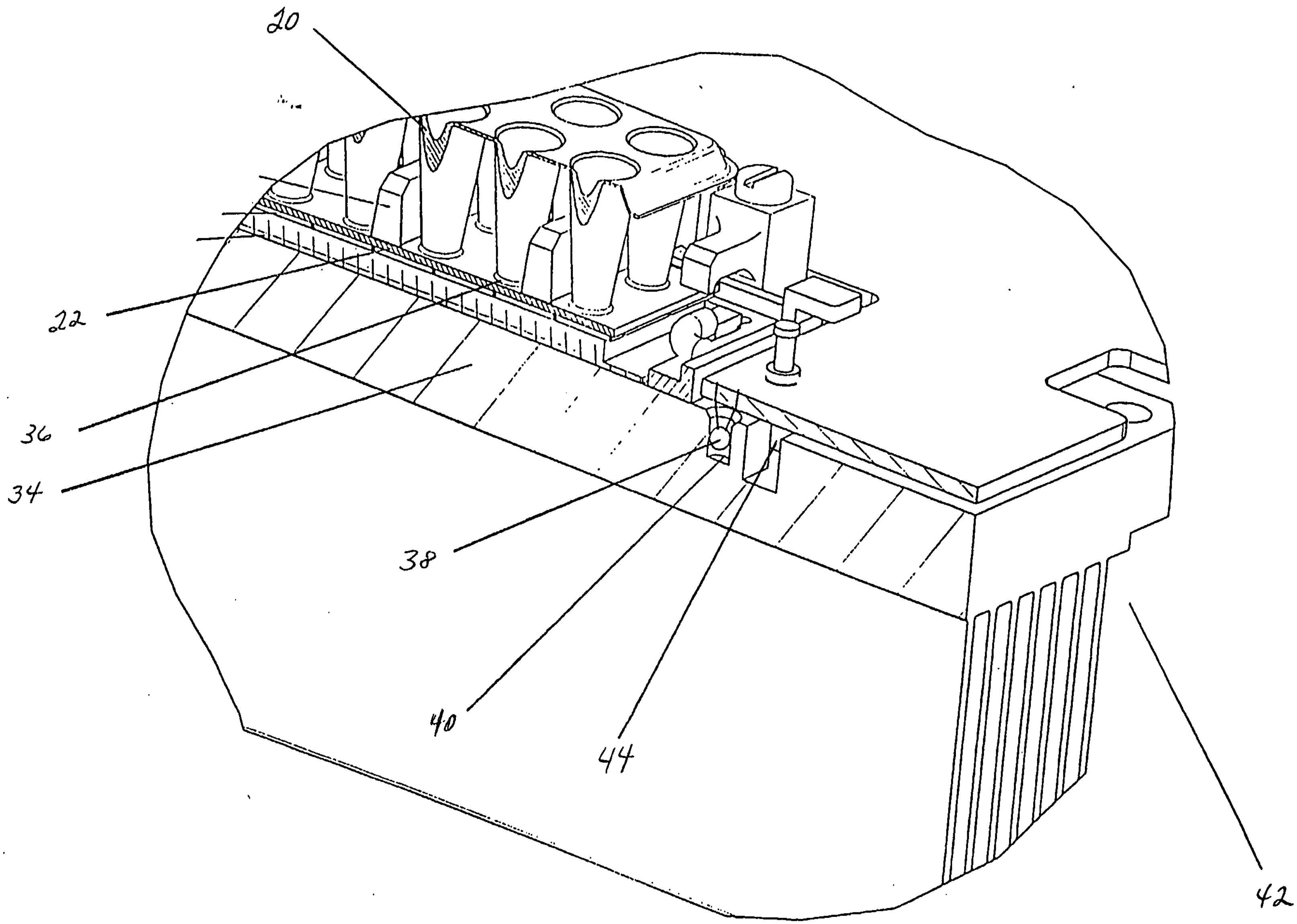


FIGURE 3

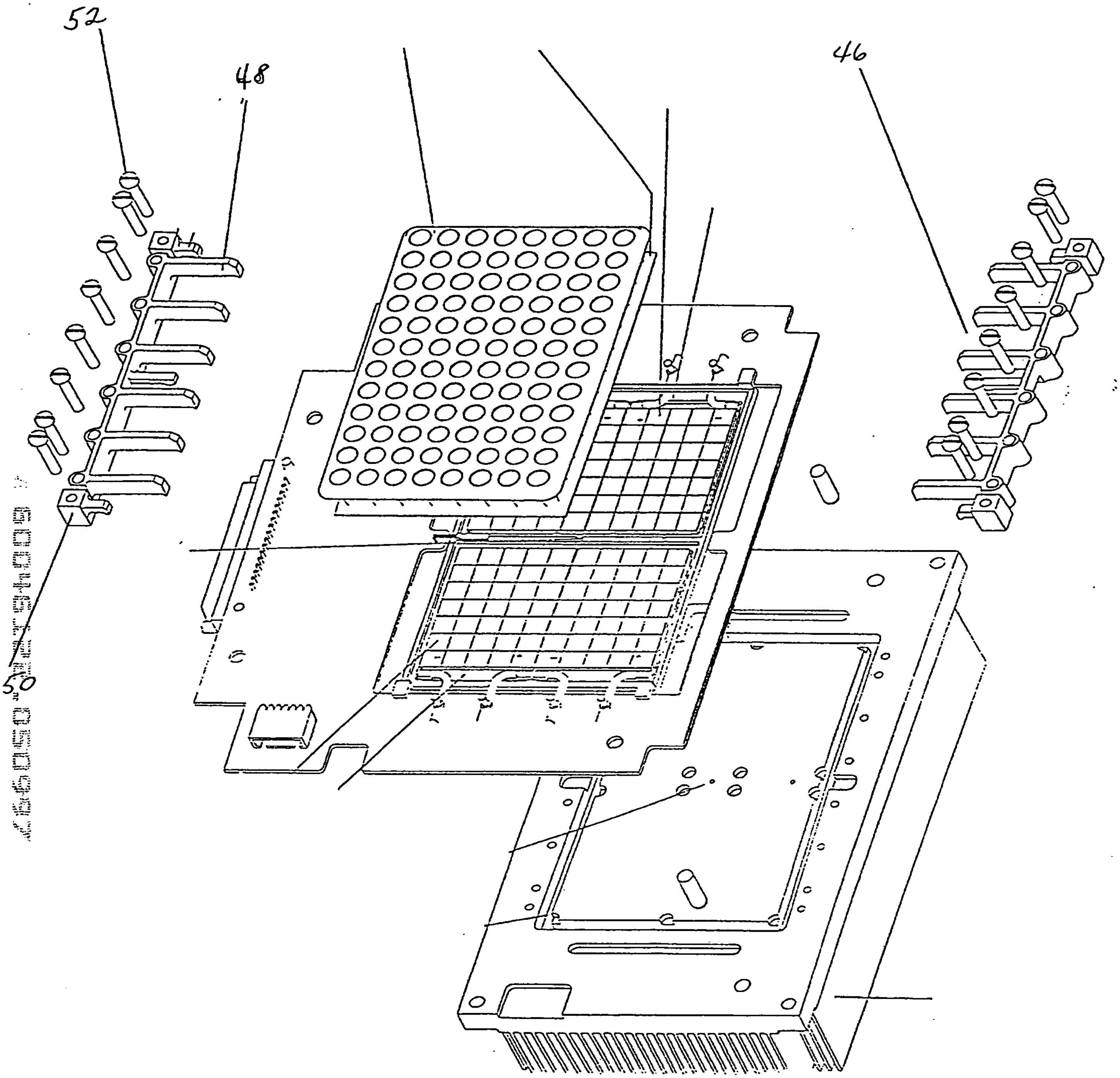


FIGURE 4

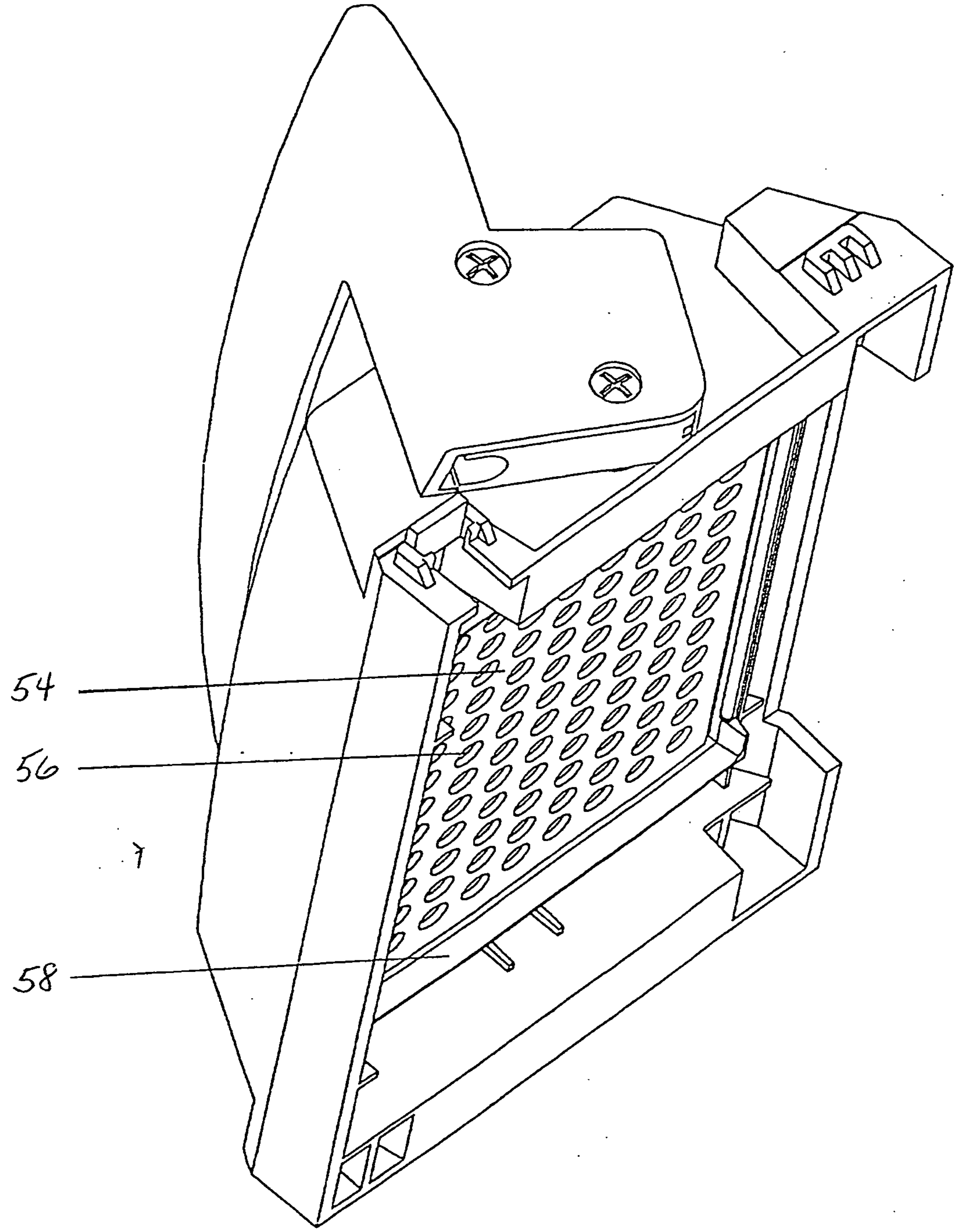


FIGURE 5

172g Silver Block, Fab Fin Heat Sink, Thermalcote II Grease, 50uL Samples.

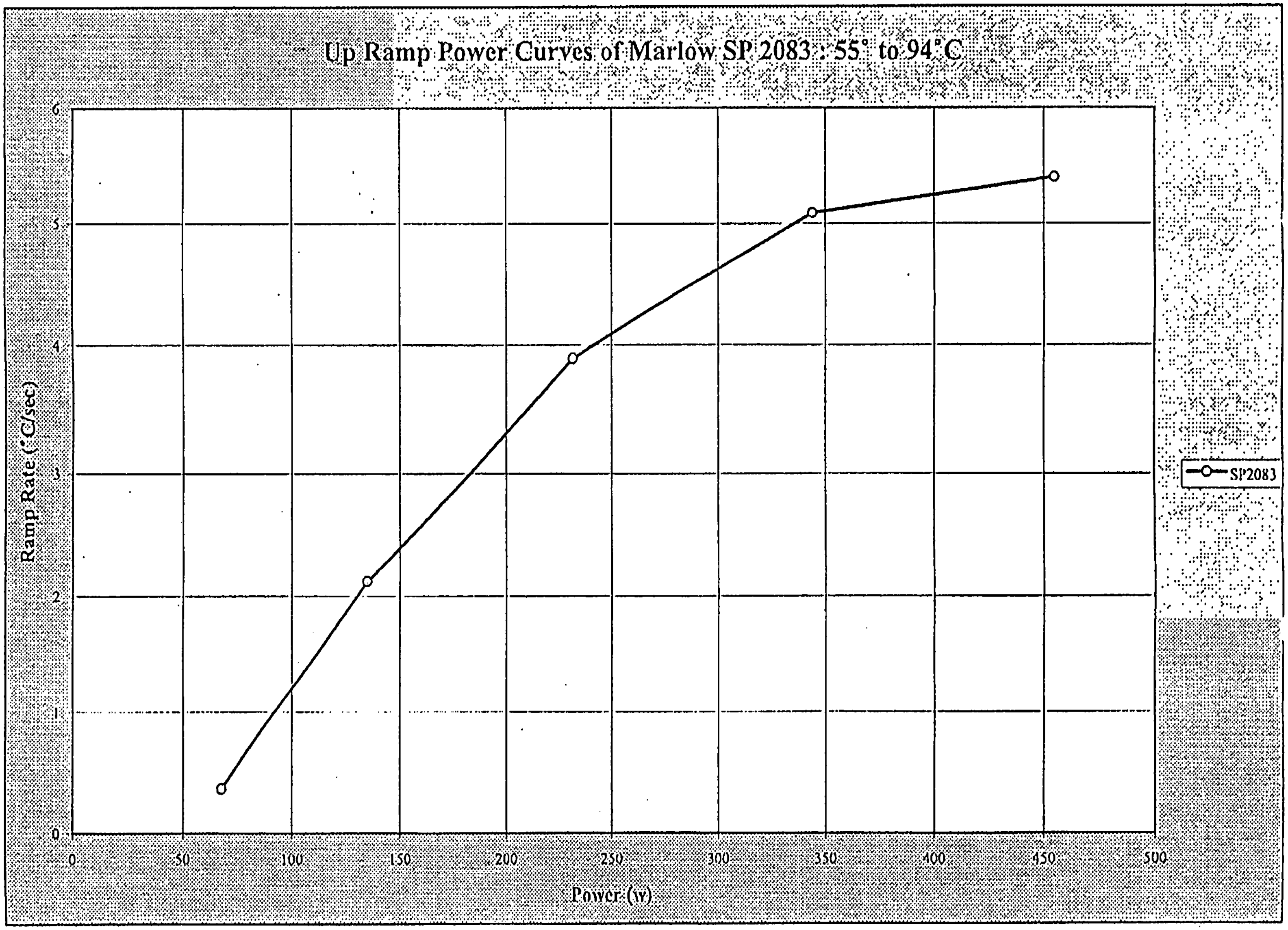


FIGURE 6

Down Ramp Rates with 172g silver block, Thermalcote II, 50uL samples, heated cover at 105±5°C

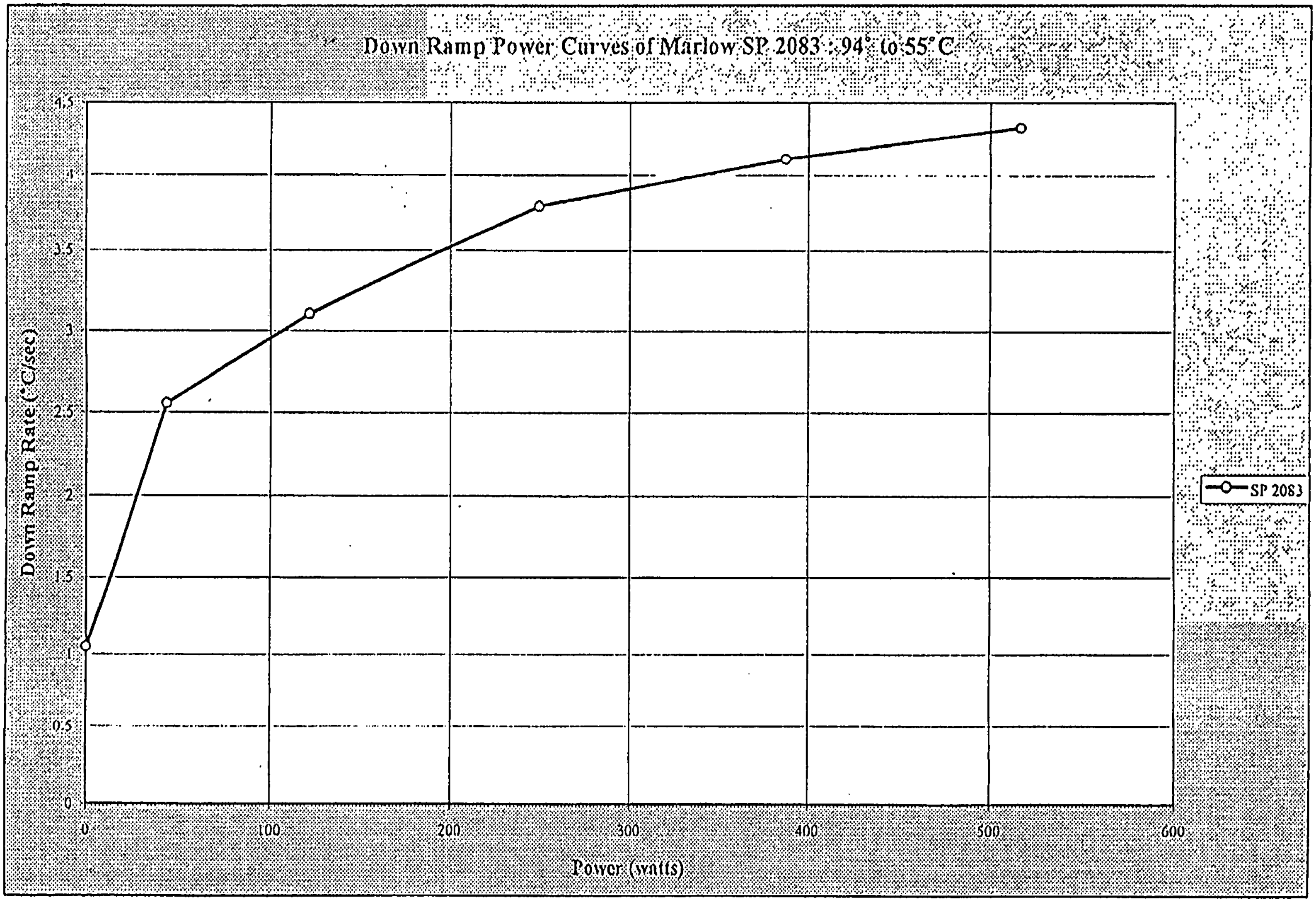
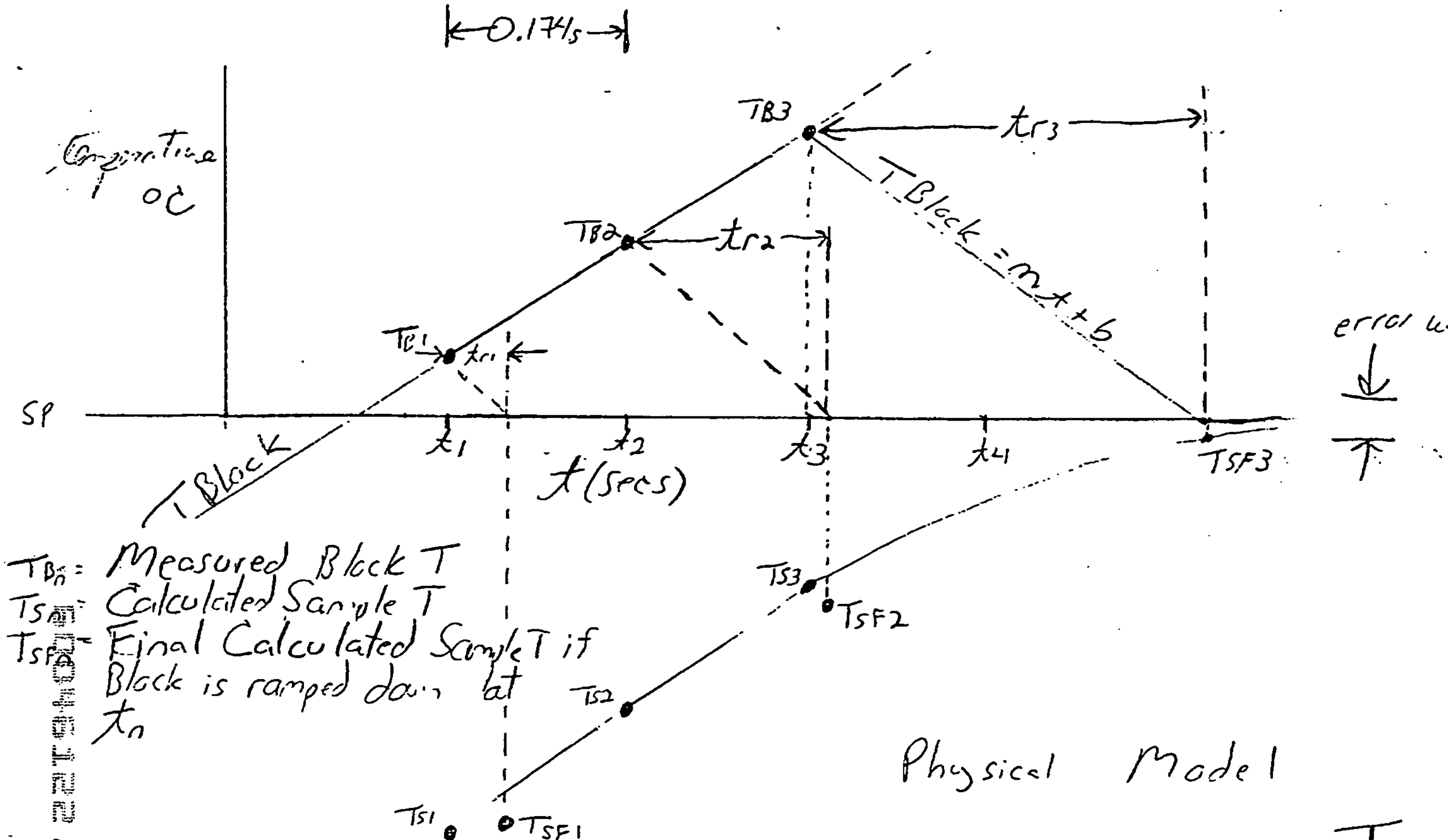
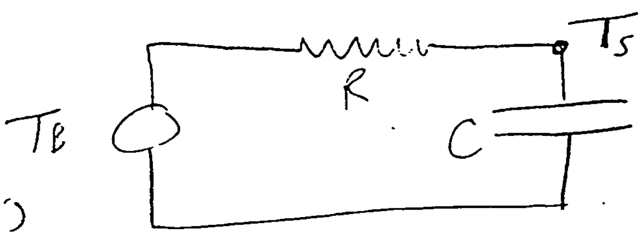


FIGURE 7



Physical Model



Every 0.174 s do the following

1 T_{Bn+1} = block T measurement

2 $T_{Sn+1} = T_{Sn} + (T_{Bn+1} - T_{Sn}) * 0.174/RC$

3 $T_{SFn} = (T_{Bn} - T_{Sn} - mRC)(1 - e^{-t_{rn}/RC}) + m t_{rn} + T_{Sn}$

4 if (T_{SFn} in error window)
then ramp block back towards SP at rate m
else
Continue ramping away from SP

When ramping back towards SP, turn ON PID control when TB in er

FIGURE 8

60046122.050992

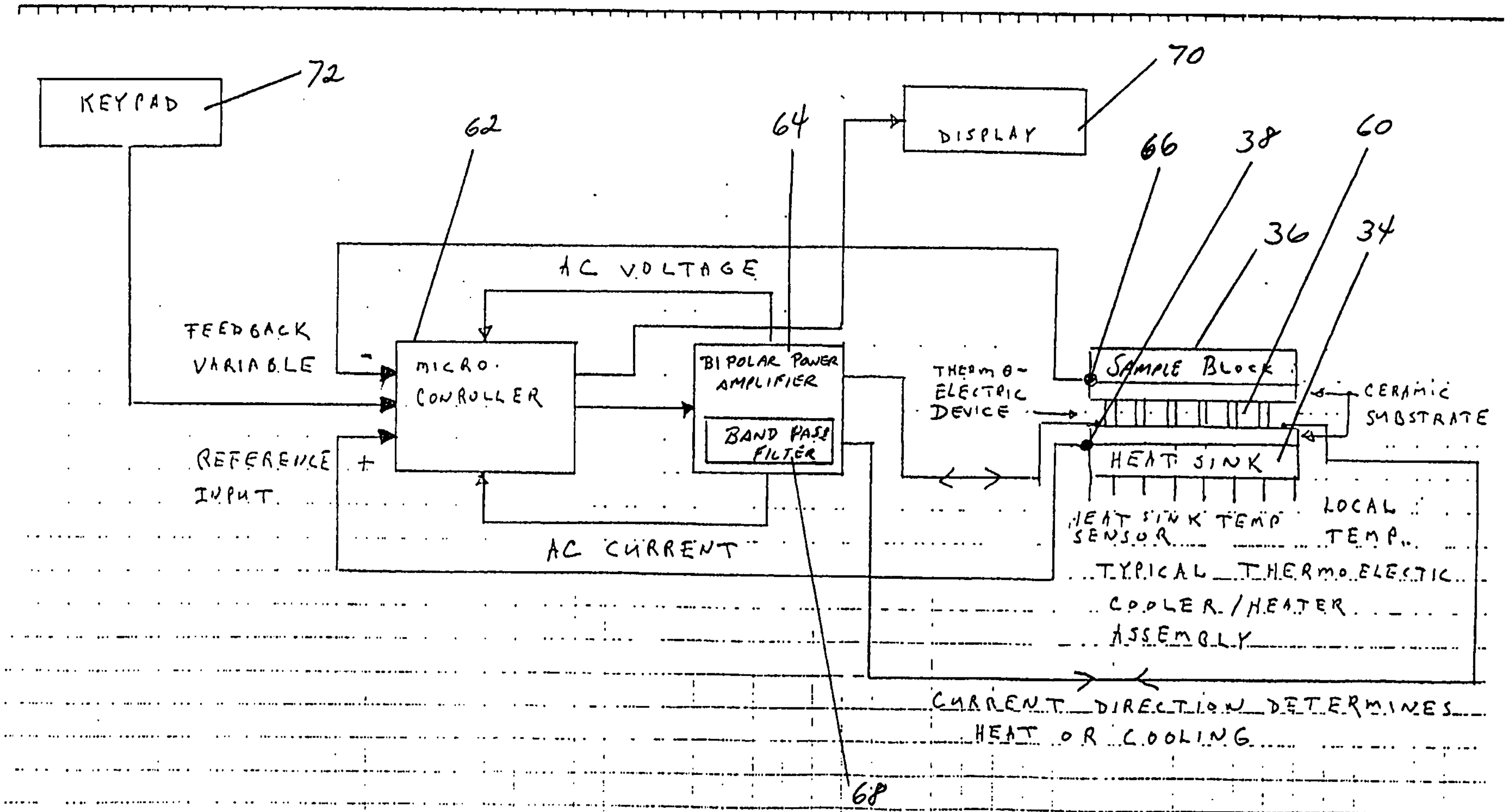


FIGURE 9

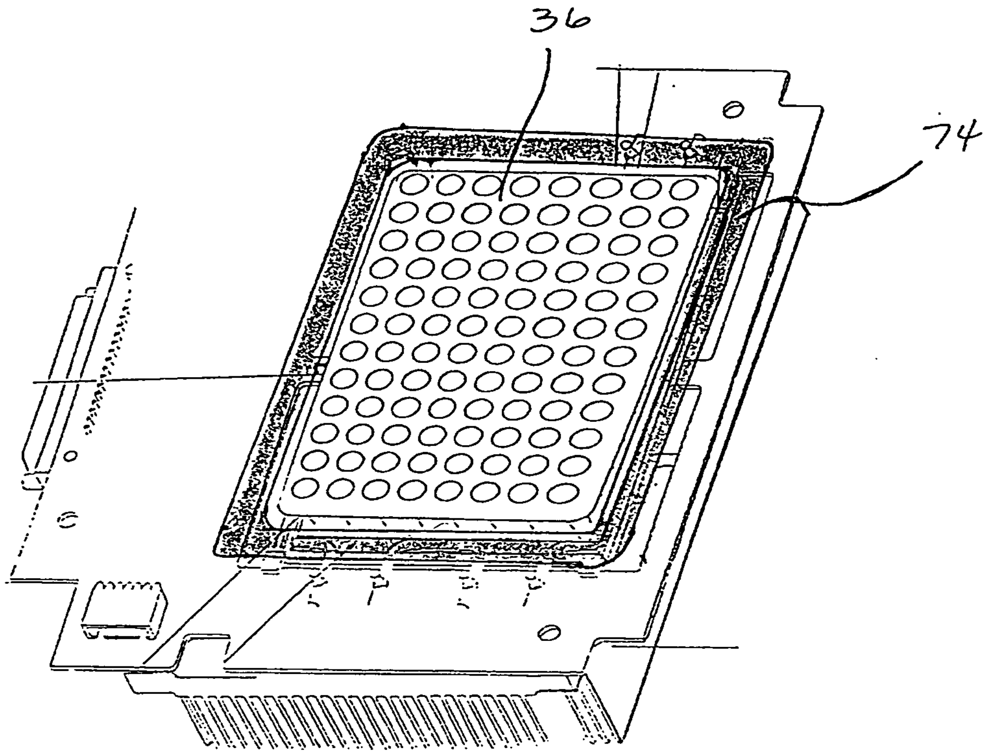


FIGURE 10

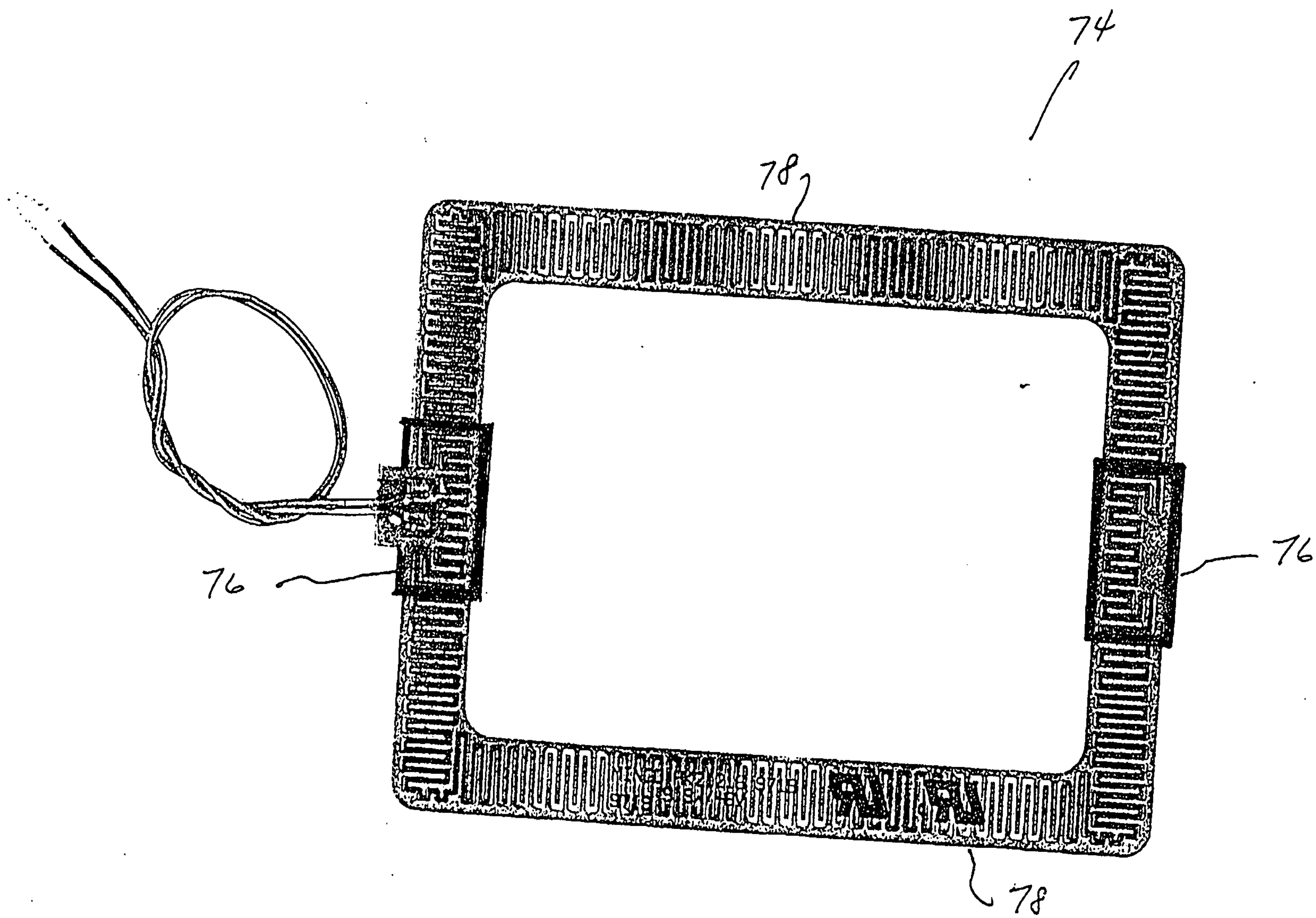


FIGURE 11

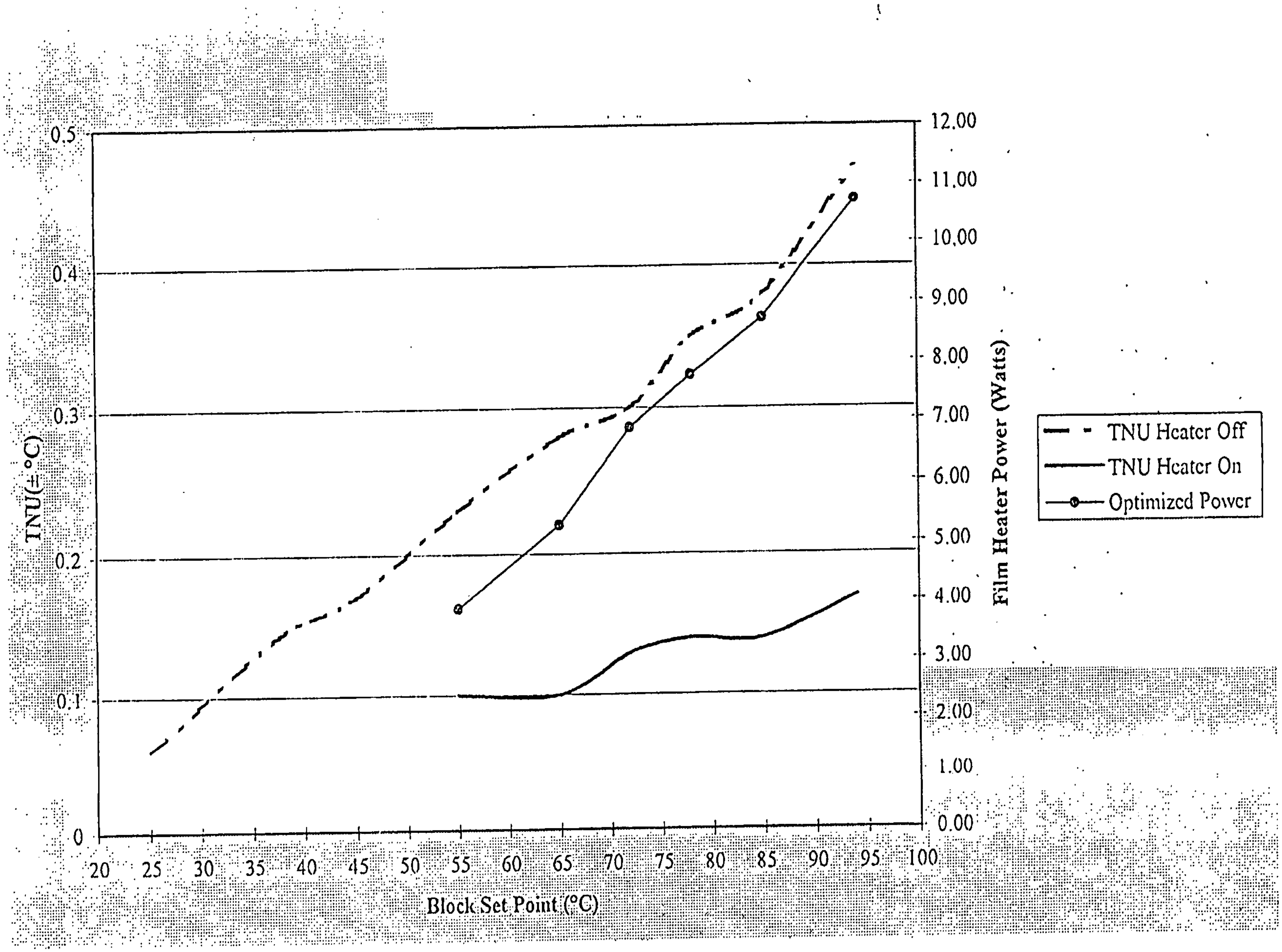

TNU(± °C)
0.5
0.4
0.3
0.2
0.1
0
Film Heater Power (Watts)
12.00
11.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
Block Set Point (°C)
TNU Heater Off
TNU Heater On
Optimized Power

FIGURE 12